CLINICOPATHOLOGICAL STUDY OF CRYPTORCHID TESTIS

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CERTIFICATE

Certified that the dissertation entitled "**CLINICOPATHOLOGICAL STUDY OF CRYPTORCHID TESTIS'** is the original work undertaken by **Dr. R. ARUNKUMAR** under our guidance and supervision, in the Department of Paediatric Surgery, Institute of Child Health and Hospital for Children, Madras Medical College, Chennai-3, during the period of his post graduation in M.Ch. Paediatric Surgery from 2005-2008, in partial fulfillment of the university rules and regulations for the award of M.Ch. degree.

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DECLARATION

I declare that this dissertation entitled **"CLINICOPATHOLOGICAL STUDY OF CRYPTORCHID TESTIS"** has been conducted by me at the Institute of Child Health and Hospital for Children. It is submitted in part of fulfillment of the award of the degree of M.Ch (Pediatric Surgery) for the August 2008 examination to be held under the Tamil Nadu Dr. M.G.R. Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

Dr. ARUNKUMAR

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HISTORICAL PERSPECTIVE

The importance of a descended testis has been known since ancient times, but the mechanism of descent remained obscure until 1786, when **HUNTER** dissected the human fetus and found the intraabdominal testes connected to the inguinoabdominal wall by a ligament called **gubernaculum** appearing to guide the testes to the scrotum.

Although **cryptorchidism** has been studied intensively both experimentally and clinically during the past century, the cause of the condition remains poorly understood. Moreover, despite surgical advances in the technique of orchiopexy, the individual management options, especially for non-palpable testis remains controversial.

The clinical issues related to the etiology, diagnosis, histopathological changes and the management of cryptorchidism are

the focus of attention during the current decade as it has a profound bearing on the future fertility potential of the individual.

INTRODUCTION

Cryptorchidism contributes the most common genital problem, and one of the most common overall problem encountered in pediatric surgical practice. Cryptorchidism literally means '**hidden testis**'. The term is derived from greek word '**Kryptos**' and '**Orchis**' meaning '**hidden**' and '**testis**' respectively, and refers to the absence of testis from the scrotum.

Although some interchangeably call a testis '**cryptorchid**' or '**undescended**', the terms are not synonymous, because cryptorchid testis may also be '**ectopic**', or '**absent**', while undescended testis typically testifies a testis which is arrested anywhere in the normal course of its descent from the intraabdominal position to the scrotal position.

Normal and abnormal testicular descent

Testicular and epididymal descent is believed to be necessary for most mammals and man to produce a fertile ejacutate; the 2-3 degrees cooler temperature provided by the scrotum appears crucial in this regard.

The process of testicular descent can be divided into 3 phases:

1) **Transabdominal migration (8-15 Weeks)**

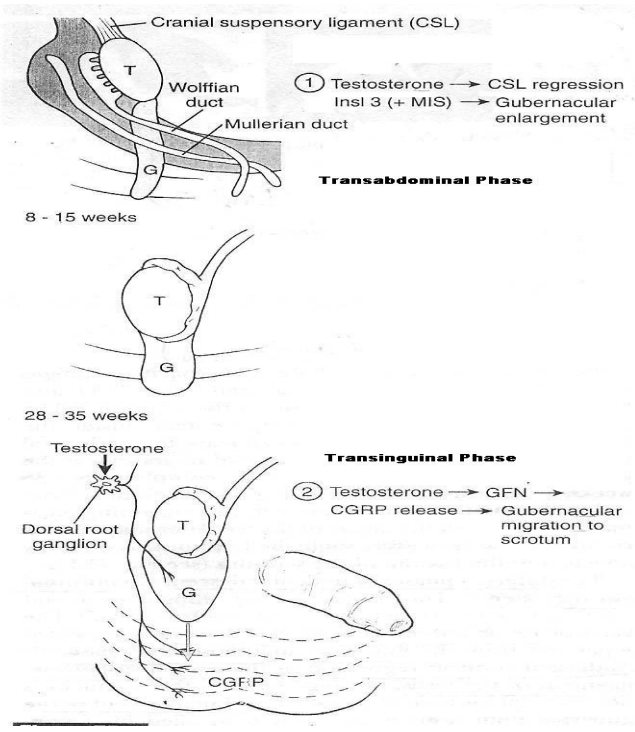
   The cranial suspensory ligament regresses under the influence of androgen testosterone, and the gubernaculum proliferates and swells to guide the testis further down. The result of transabdominal migration in humans is testicular position at the internal inguinal by the 12^{th} week of gestation.

2) **Process Vaginalis: (15-24 Weeks)**

During the 3rd month of gestation the processus vaginalis grows along the gubernaculum and extends from the peritoneum into the inguinal canal and scrotum bringing with it the testis further down. The testis does not change position between 3 and 7 months of fetal life.

3) **Transinguinal descent (24-28 weeks)**

This occurs very rapidly. The testosterone acts on the **genitofermoral nerve** causing the release of **Calcitonin gene related peptide (CGRP)**, causing the regression of gubernaculum and the migration of the testes to the scrotum. Normally the processus vaginalis closes completely before birth, but when the testis is undescend, the processus vaginalis remains patent .



Definition and Classification:

Several existing definition and classifications attempt to communicate the physical findings related to cryptorchidism. Clearly the two most significant categories include :

- **Palpable : 80%**
- **Non Palpable : 20%**

Palpable :

True undescended testis : 75%
Ectopic testis : 5%

Non Palpable :

Intrabdominal	: 60%
Inguinal	: 20%
Absent testis	: 30%

Incidence :

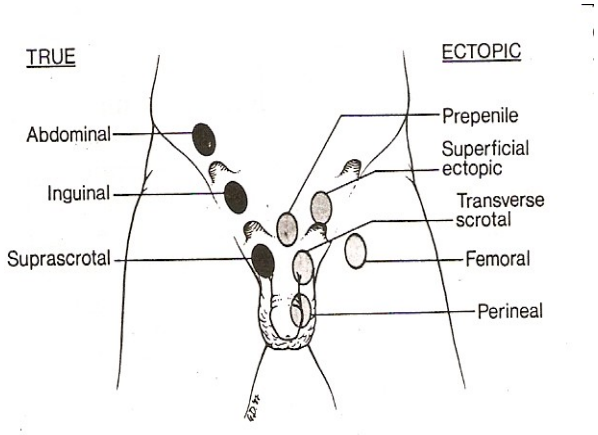
Premature	: 33%
Full term	: 3%
1 year	: 0.8 – 1 %
Unilateral	: 68%
Bilateral	: 32%
Right Side	: 70%
Left Side	: 30%

RETRACTILE TESTIS:

These are often misdiagnoised as undescended testis, and will not be entertained in this category. The retractile testis can generally be manipulated into scrotum, where it will remain momentarily until restimulation results in retraction into the groin due to a cremasteric reflex. Differentiation of the retractile from the true undescended testis is not always easy, and the diagnosis of a retractile testis is occasionally made on a examination under anesthesia.

ECTOPIC TESTIS:

This is a normally developed testis with only an abnormal migration. These are not in actual terms included under the term Undescended testes. The most common site of ectopia is the superficial inguinal pouch of Denis Browne, They are also found in positions like femoral, pubic, penopubic, and perineal positions.



VARIOUS TESTICULAR LOCATIONS

ETIOLOGY:

1. Pituitary – gonadotropin deficiency

2. Primary Testicular abnormality

3. Anatomical abnormalities

4. Dysgenetic testes/Regression syndrome

This study is done to find out the histological changes in cases of **UNDESCENDED TESTIS** to give a prognostigation of future fertility and the risk of developing future malignancy in the form of 'intratubular germ cell neoplasia'.

LITERATURE REVIEW

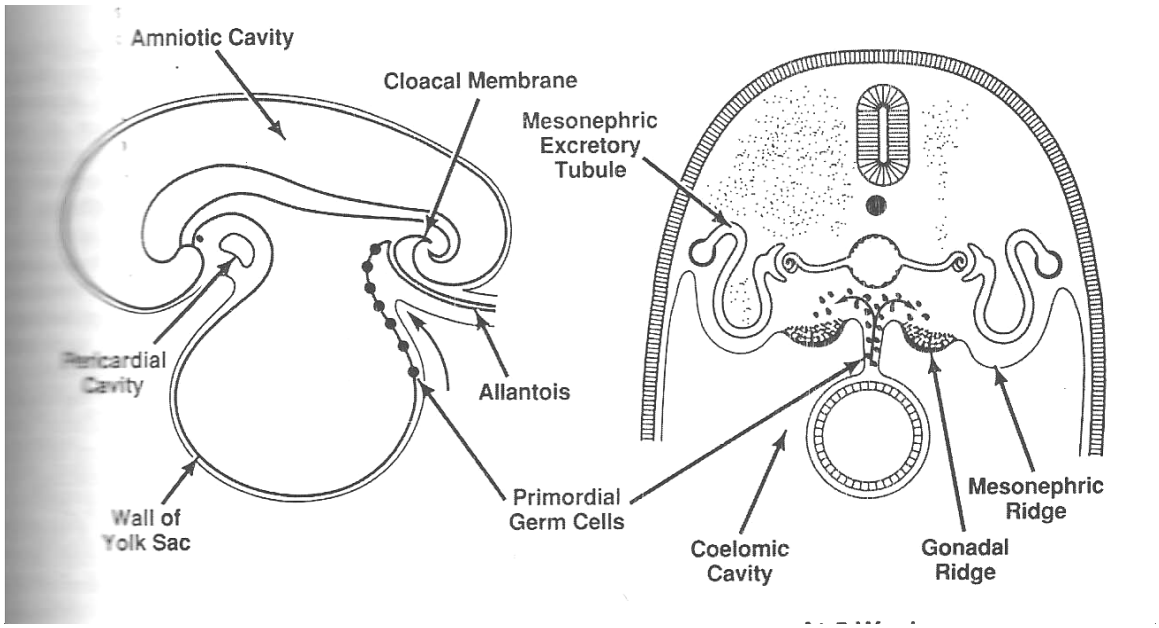
An understanding of normal testicular development is helpful to the diagnosis and management of cryptorchidism. Normal male phenotype is the result of a cascade of gene activations and hormone-receptor interactions that are tightly regulated temporally and spatially in the embryo.

Embryogenesis:

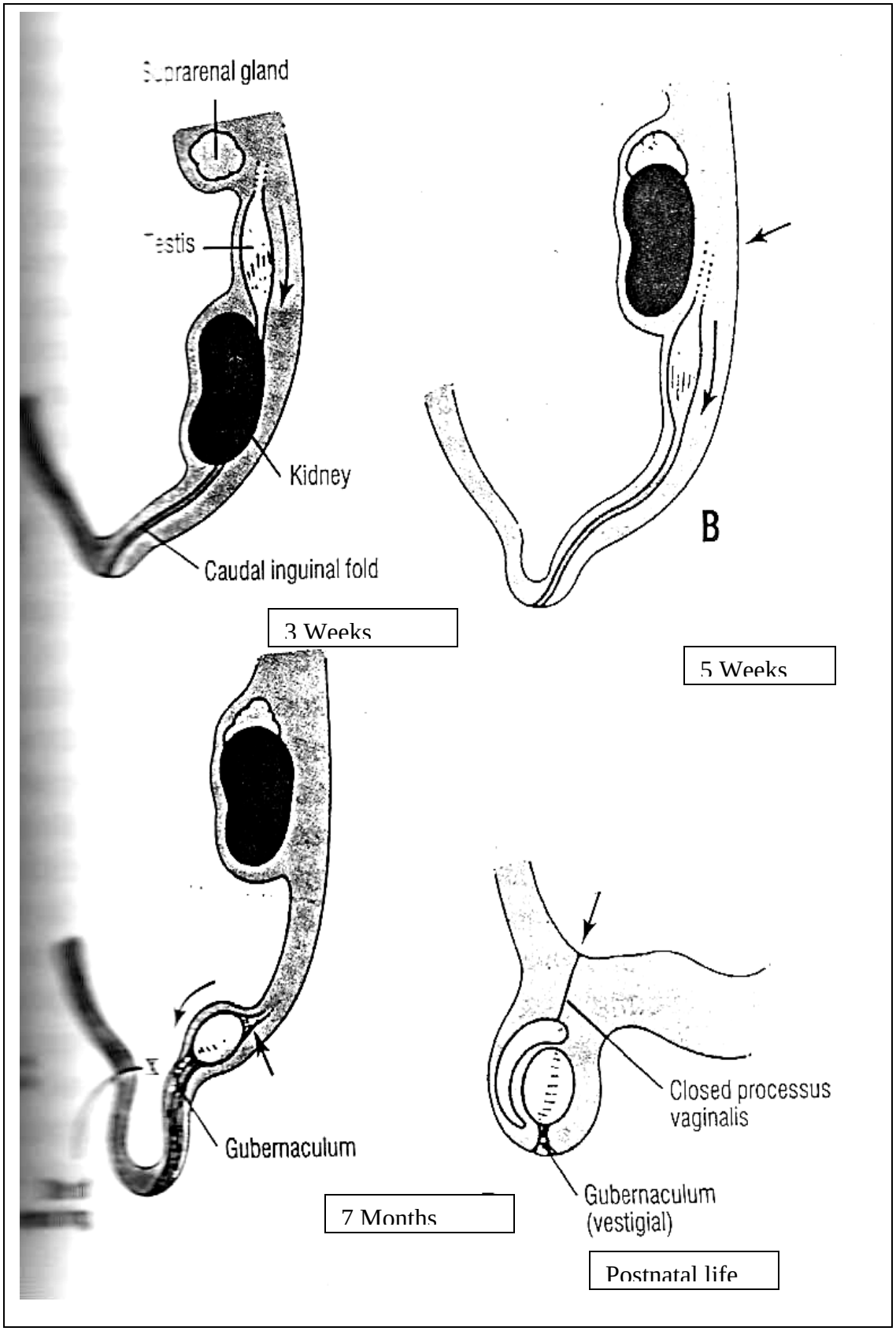
This begins in the 5th week of gestation, when proliferation of coelomic epithelium and the underlying mesenchyme medial to mesonephros produces the bipotential gonad. Formation of this bipotential gonad is dependent on such genes as the Wilm's Tumour gene (WT-1) and Steroidogenic factor (SF-I).

Epithelial primary sex cords then grow into the underlying mesenchyme, resulting in the development of a cortex and medulla. The fate of the bipotential gonad is determined by the presence or absence of a normal SRY (Sex determining Region-Y linked) gene situated in the short arm of Y chromosome.

Under the activation of SRY and other downstream testis determining genes, the cortex regresses and the medulla develops as testis, beginning in the 6th week of gestation. Primordial germ cells situated in the wall of the yolk sac from the 4th week of gestation migrate along the dorsal mesentry of the hindgut to reach the gonad by 6th week gestation. Sertoli cells appear by wk 6, and the Leydig cells appear by wk 8 to produce **MULLERIAN INHIBITING SUBSTANCE** and **TESTOSTERONE** respectively (Jirasek)$_{)(1)}$.



Migration of the Germ Cells from the Yolk Sac to the Gonadal Ridge



Testicular Descent

To achieve the normal male phenotype, it requires normal hormonal and its receptor function, quantity, location and timing. Given these the Mullerian Inhibiting Substance causes involution of the ipsilateral mullerian duct and the testosterone promotes masculinization of the ipsilateral Wolffian duct to provide normal male internal and external genitalia by wk 10-13 of gestation.

Development of male external genitalia including the scrotum, occur between wk 10 and 15 of gestation, and results from the conversion of testosterone to dihydrotestosterone by the enzyme 5α reductase type 2 in these tissues.

Proper development of the scrotum is important because it enables the testis to migrate outside of the body and reside in an extracorporeal environment conducive for its growth.

Postnatal development:

After birth, continued changes occur in the various cell compartments of the testis. Initially the primordial germ cells differentiate into **gonocytes**, lying adjacent to the basement membrane,

and then into **fetal spermatogonia**, which remains postnatally and can be seen through the 7th year. Postnatally between the 3rd and 5th month after birth, they transform into **Type A spermatogonia**, then into **Type B spermatogonia** by 4 years of age, as normal steps in the maturation process.

The germ cell undergo a quiescent period until puberty, with the onset of spermatogenesis. At this time the spermatogonia transform into **Primary spermatocytes,** which duplicate their DNA content and then undergo meiotic division (transformation) into **Secondary spermatocytes**, with the haploid number of chromosomes.

Spermatids develop subsequently with the development of puberty, ultimately differentiating further into **Spermatozoa**. Puberty onset occurs in most boys by 13 years of age, and is completed in most by 15 years (Hadziselimovic, 1987)$_{(2)}$.

Simultaneous changes in **Leydig** cells are present at birth, and are observed through the first 4 months, when their number decreases and their transformation into '**juvenile**' variety begins. No further changes

occur until puberty when it becomes '**adult**' variety. The increase in number and histological activity are coordinated temporally with increase in testosterone secretion produced by them. Leydig cells, in addition to producing testosterone, also contain **Aromatase** , **NO synthase**, **Substance P**, as well as **Methionine-enkephalin** immuno reactivity.

Sertoli cell changes in developing testis are now more fully understood, and are seen lining the seminiferous tubules. At birth, they number 40±3-4 /tubule, and at puberty 10±1-2/tubule. Although their numbers per tubule decreases with aging, their size increases 8-10 times by puberty, concomitant with increase in tubular diameter. Histologically, sertoli cells can be distinguished into **Sa, Sb** and **Sc** (adult) in addition to **Sf** (fetal) forms as puberty progresses, as normal steps in the maturation process. The **Sf** variety is believed to secrete **Mullerian Inhibiting Substance** (JOST, 1970)(3).

The functions of the **sertoli** cells have only recently been recognized and it is obvious that the **sertoli** cells are not a passive

structure. Junctions between **sertoli** cells are among the tightest in the body and divide the seminiferous tubule into an outer '**basal**' compartment containing **spermatogonia** and **pre-leptotene spermatocytes**, and an inner '**ad luminal**' compartment containing the more mature forms. Junctions develop at puberty and define the '**blood testis**' barrier, which gives the ad luminal compartment a protective environment.

Human **sertoli** cells, in addition to producing **MIS**, also secrete ABP (androgen binding protein); **Inhibin**, which suppresses FSH secretion; and other substances like **TGF-X; Transferrin; CPK; Testibumin; LDH; Alk PO4** and **γGGT**.

Aromatisation of **testosterone** to **estradiol** occurs in the **sertoli** cells under FSH control. **Sertoli** cells also have **phagocytic** action and **spermatogenesis regulating capacity**. Thus **sertoli** cells have important effects on differentiation, spermatogenesis and endocrine function.

Although earlier studies indicated that testicular development is static during the first years of life, stereologic studies showed progressive increase in testicular volume from birth to adulthood. This increase is not sufficient during the first 5-6 years to produce distinctive changes by light microscopic study.

In Muller and Shakkeback study (1978)[(4)], mean tubular diameter was constant through year 5, with slight enlargement through year 14, and a significant increase in tubular diameter between 14-18 years old. The average mean tubular diameter is 80-90 microns.

The numeric density of germ cell nuclei per tubule increased progressively from age 5, as did the total number of germ cells. **Seminiferous tubules** occupied about half the testicular volume before puberty and about 67% from 14-18 years. By 8 years **primary spermatocytes** may be evident. Development becomes progressive thereafter. **Sertoli** cells become more mature with increased cytoplasm and oval, often nucleolated nuclei.

Spermatogenesis and spermiogenesis may be seen by year 11. **Leydig** cells, usually not evident histologically after 4 months, reappear as soon as 8 years and more numerous at 11 to 14 years.

After spermatogenesis has been completed, the testis resembles the adult testis. Changes related specifically to advancing age are not readily discernable in an individual testis.

After age 40 years, **LH & FSH** increase, and serum **Testosterone** values slowly decrease. Weights of the testes are similar in young and older men, but the percentage of total testicular weight represented by the tunica albugenia and the thickness of tunica albugenia are greater in older adults. The weight of the testicular parenchyma is similar or reduced in older, but the daily sperm production per testis and per gram of testis is greater in young men.

With increasing age there is reduced volume of **parenchyma**, **seminiferous tubules**, and **seminiferous epithelium**; in addition the number of **sertoli** cells are reduced per gram, per testis and per serminiferous tubule. **Johnson** and **Kaber** (1968)$_{(5)}$ described reduced

number of **leydig** cells and **myoid** cells as age progresses. **Honore** (1967)[6] however, indicated that there is **leydig cell hyperplasia**, but this was not assessed in a quantitative fashion. He also described tubular sclerosis, focal mononuclear cell infiltration, capsular smooth muscle hyperplasia, and dilation of rete testis. **Johnson**(1968)[5] also described increased thickness of myoid cells, and extracellular compartment as age progresses, but no difference in volume density of myoid cells, and he interrupted the changes as secondary to reduction in tubular length.

In the humans, in general, **spermatogenic** and **hormonal** function do not abruptly cease. It is difficult to determine the causes of various changes, but decrease in hormonal function is probably at least partly due to loss of about 8 million **leydig** cells per decade.

Reduced function and altered structure of the testis as age progress have multiple etiologies, including arteriosclerosis, reduced nutrition and intercurrent disease.

Normal development of the Hypothalamic-Pituitary-Testicular axis.

During the 8th fetal week, the testes begins secreting **testosterone** under the stimulation of **Maternal Chorionic Gonodotropin** hormone, thereby influencing development of internal ducts and external genitalia, and perhaps testicular descent. Subsequent activation of fetal **Hypothalamic-pituitary-testicular** axis results in continual of gonadotropin stimulation of testosterone production (**Smail Etal**, 1981)(7)

At term, and during the first 6 months after birth, **LH, FSH** and **Testosterone** are elevated (**Forest etal**, 1973)(8), Thereafter all the three fall progressively, except for a small peak in **LH** activity noted between 4 and 6 years of age, paralleling the appearance of well developed '**juvenile**' **leydig** cells and **primary spermatocyte** (**Waaler,** 1979)(9)

Puberty onset marks the next notable event; with decreasing sensitivity of the **gonadostat**; increased **Gonadotropin Releasing Hormone** (GNRH) release and '**pulse synchronized**' increase in **LH** and **testosterone**, and a lesser increase in **FSH**, (**Boyar et al,** 1974)[10] **leydig** cell numbers and activity increase, **sertoli** cells undergo completion of maturation and spermatogenesis also become fully complete. These events illustrate the synchronous interaction of the hormonal and cellular changes occurring at this time.

Abnormal Hormonal findings:

Characterization of hormonal profiles in **Cryptorchid** boys of varying ages has done much to aid our understanding of the condition. Some studies have identified abnormalities in **hypothalamic-pituitary-testicular** axis. These findings are the more revealing, and lend credence to the belief that this problem has a hormonal basis.

Normal boys with descended testes demonstrate a transient rise in testosterone levels, peaking at 60 days after birth. This physiololgic

occurrence has been described as a '**marking phenomenon**', eventually allowing for normal function in male organs, possibly including spermatogenesis, later in life. In both unilaterally and bilaterally cryptorchid boys, a '**blunted**' response occurs, and the **basal testosterone** levels are all lower than in normal boys (**Gundral et al**, 1978). In addition, a **blunted testosterone response** to HCG stimulation is observed in about one third of those children.

There is also a defective **LH** secretion present early in life in some **cryptorchid** children, suggesting that early delay in **LH** secretion may be responsible for the abnormal testosterone response at 60 days, and may relate to abnormal descent. As these findings are noted both in unilateral and bilateral **cryptorchids**, a primary decrease in **LH** secretion rather than a primary **leydig** cell defect appear to be the cause. Because it appears so early in life, an acquired **leydig** cell defect secondary to abnormal testicular position does not appear to be likely. Other studies involving large number of infants have failed to confirm these hormonal abnormalities, in part may be related, to the **heterogenous** nature of **cryptorchidism**.

Those with reduced **LH** levels, in general, have the lowest germ cell count (**Hadziselinovic)** [21]. These reports indicate that in some patients, the early **LH-testosterone** defects persists throughout puberty. By puberty, testosterone response to **HCG** normalizes, and most cryptorchid boys undergo normal puberty. Mean **FSH** levels in cryptorchid children are usually normal.

Adults with **bilateral cryptordism** and late treated bilateral cryptorchidism are virtually always sterile, and may undergo **premature androgen failure**, ie in their mid 40's (**Amelar**, 1966)[12] These observations indicate the **progressive** nature of this disease process.

Histology and ductal development in cryptorchids:

Histological appearance of **cryptorchid** testis is completely different from that of a normally descended testis. Whereas the later undergoes age dependent, progressive development the undescended testis is constantly retarded.

Cryptorchid testis in the adults are uniformly devoid of germ cells as age progresses. Their tubular diameter is usually diminished, and their basement membrane are thickened. and sometimes **hyalinized**. **Sertoli** cells are prominent often appearing as a '**Sertoli cell only'** pircture. The **leydig** cells appear **relatively hyperplastic**, and marked **interstitial fibrosis** often occurs.

These changes are already evident at puberty, the most obvious being related to the onset of spermatogenesis. Germ cells at this time normally develop the capacity to progress through a series of **mitotic** divisions (**Spermatogonia**), **meiotic** divisions (**Type A and Type B** spermatocytes, morphologic transformation from round to elongated forms (**Spermatids**), and finally release **Spermatozoa** into the tubular lumen.

Prior to these events, the germ cells in the prepubertal testes are of the **prespermatogonial** type. Distinction between **prespermatogonia** and **spermatogonia** is important because only with

spermatogonial formation can the process of **spermatogenesis** be initiated.

Cryptorchid testis at puberty are variable in appearance, but definitely demonstrate abnormalities of germ cell morphology and number. **Spermatogenosis** is uncommon, with the exception in the more distally situated testis, and appear arrested in the **prespermatogonial** and **spermatogoinal** level (**Gondas etal,** 1982)[13]. Germ cells numbers remain diminised. **Leydig** cells are usually reduced in number and appear **atrophic** when examined by electron microscope and volumetric analysis. Despite these observation **testosterone** production is adequate to initiate and maintain normal puberty in most cases, even in **bilateral cryptorchid** individuals, attesting to the functional capability of these **leydig** cells at this age, regardless of their appearance (**Duckerman et al** 1979).

Histological analysis of **cryptorchid** testes before puberty has provided the most insight and benefit in understanding their effect on fertility. These observation, in particular, have resulted in a dramatic alteration in the recommended age at orchiopexy. In an article in the

International Journal of Urology 2007, Park KH et al made a study to determine the optimal timing for **orchiopexy** and concluded that to protect future fertility potential **orchipexy** be performed no later than 2 years of age in patients with palpable inguinal testis.

As early as 1929, it was noted that the younger the age an **undescended** testis is examined, the more closely it resembles the histologic appearance of the descended testis (**Cooper** 1929)$_{(16)}$. Within the first months of life, the number of germ cells in **cryptorchid** testis and **descended** testis is equal, but by the end of the first year, however differences already exist. Although, the mean number of germ cells in both is equal, a wide standard deviation exists among **cryptorchid** testis, implying that same already have diminished germ cells (**Mengel etal** 1974)$_{(17)}$. The number of **spermatogonia** does not increase as in descended testes, although the total number of germ cells remain normal (**Hadziselimoric**)$_{(17)}$ Failure of gonocyte transformation has been implicated, paralleling a reduction in **leydig** cell number.

The scrotal testis in an **unilateral cryptorchidism** has as a rule, more germ cells than **cryptorchid** one, but fewer than age matched descended testis.

In upto 40% of **unilateral cryptorchid** boys, the combined germ cell count of both testes do not exceed that for **bilateral cryptorchidism**, indicating a deficiency in both testes, and some of those testes are already devoid of all germ cells as early as the first year of life (**Hedinger** 1979)[18]. In general, the reduction in germ cells in the descended testes is directly proportional to the severity of reduction in the **cryptorchid** one. This provides the basis for **Testicular dysgenesis/Regression syndrome**.

In addition to **age** and **gonadotropin** level as the significant factors correlating directly with **histologic** abnormalities in **cryptorchidism**, there is a direct relation between testes **location** and **germ cell count**.

Although **intraabdominal** testis within the first year have a normal number of germ cell number, 90% demonstrate complete loss of germ cell by puberty. Only 41% of **inguinal** and 20% of **penoscrotal** testes undergo similar loss (**Hadziselimovic**). Structural abnormalities

of the **ductal** system associated with **cryptorchidism** also occur more frequently in higher **undescended** testis. Grossly identifiable lesions of the **vas deferens** and **epididymis** occur clinically in atleast 1/3 of **undescended** testes, especially in an **intraabdominal** position. These are characterized by various degrees of detachment between the **epididymis** and **testis** and by elongation and looping of the caudal **epididymis** and **vas**. An elongated or extended **epididymis** is the most commonly encountered **ductal** abnormality associated with **cryptorchid** one.

Whereas gross detachment may be clinically obvious; microscopic areas of **agenesis** or **atresia** may also occur (**Kroovand** and **Perlmutter**, 1981)[(19)].

The more severe the **ductal** abnormality, the more severely reduced is the total germ cell count in the associated **cryptorchid** testis (**Gill etal**, 1987). Although the significance of these **ductal** abnormalities with regard to sperm capacitation and transport is uncertain, in addition to correlating with **cryptorchid** testis histology,

their impact on surgical correction of the **cryptorchid** testis is very clear.

When the testes is not evident upon inguinal exploration, the presence of a patent process vaginalis suggests that an **intraabdominal** rather than a '**Vanishing testis**' may exist. Intraperitoneal exploration becomes mandatory.

The findings suggest that although some cases of **cryptorchidism** are associated with anatomic abnormalities preventing descent, most appear to be associated with hormonal abnormalities that demonstrate **histologic** correlates. Primary **LH** deficiency resulting in **Leydig** cell **atrophy** and impaired **testosterone** secretion may be the cause of germ cell damage seen early on and of the **subfertility** seen in treated **cryptorchid** individuals later in life.

The severity of the abnormality does not always correlate with the degree or duration of the maldescent; although generally the more

severe abnormalities are seen in those **intraabdominal** testis detected only in later adolescence.

Clinically decreased fertility is a well known consequence of **cryptorchidism**. Even after **orchiopexy**, fertility is impaired in 50-70% of **unilateral** and 80% of **bilateral** cases; approaching 100% in **B/L intraabdominal** testes.

The most important pioneering work about **histopathology** predicting the future fertility potential and risk in the development of malignancy in the form of intra-tubular **germ cell neoplasia** has been done by **Nistal etal (1980)**[21] **(2007)**[22].

In pubertal biopsies from **cryptorchid** cases, abnormalities of testicular parenchyma were classified into 4 types, according to the **morphometric** parameters comprised by estimation of **Mean Tubular Diameter (MTD)**, **Tubular Fertility Index (TFI)** (no. of germ cells per tubule), and **Sertoli Cell Index (SCI) (**no. of sertoli cells per tubule).

The normal prepubertal testis will show a **mean tubular diameter** of **80-90 microns** and more than **90%** of the tubules showing **spermatogonia** and the number of **sertoli** cells will be **40±5 at birth**,

which gradually decreases and becomes **10±2-3 at puberty** and **adulthood,** (**Nistal etal ,1980)**[22], **Human pathology**.

The histology of **cryptorchid** testes, in addition to showing alterations in **Mean Tubular Diameter (MTD)**, **Tubular Fertility Index (TFI)**, and **Sertoli Cell Index (SCI)**, may also have the following features like **clustering of tubules**, **microcalcification**, **hyalinisation** of the basement membrane of **seminiferous** tubules, as well as abnormal shapes of the tubule, like **ring tubules**, **calcospherules** and **interstitial fibrosis**, (**Nistal et al,** 1980) [22]. In later life it may give a '**sertoli cell only**' picture.

Classification of cryptorchid testes based on morphometeric parameters.

Type	Description	MTD (µ) (Mean Tubular Diameter)	TFI(%) (Tubular Fertility Index)	SCI (Sertoli Cell Index)
I	**Slight alteration**	**70-90**	**>50**	**Normal**
II	**Marked germinal hypoplasia**	**60-70**	**30-50**	**Normal**
III	**Severe germinal hypoplasia**	**<60**	**<30**	**decreased**
IV	**Sertoli cell only picture**	**<60**	**<30**	**Immature sertoli cell hyperplasia**

Nistal etal (2007) in their study of **post pubertal** biopsies came to the conclusion that type III & Type IV lesions during the **prepubertal** period are likely to be followed in adults with testicular lesion such as **Incomplete spermatogenesis**, **Mixed tubular atrophy**, and **lesions of the basal compartment of seminiferous tubule**, foretelling a worse prognosis even for **IVF**. They, in their studies concluded, from pubertal **cryptorchid** testicular biopsies prognosis concerning fertility in adulthood can be predicted.

Malignancy and Intra-tubular germ cell neoplasia:

Individuals born with an undescended testis have an approximately **40 fold** increased incidence of testicular malignancy over those of scrotal testes.

Approximately **10%** of all **testicular tumours** develop in individuals with a **history of UDT**. The incidence of malignant transformation increases with the higher location of **Undescended estis**, with a tumour occurring **4 times** more likely in **abdominal** than **inguinal** testes.

Testicular biopsy at the time of **orchiopexy** may reveal **Carcinoma-in situ** in both the **undescended** and the **descended contralateral** testes; and is more common in the **intraabdominal** than an **inguinal** one. However such pre-malignant changes (**intra-tubular germ cell Neoplasia**) may not be seen in **cryptordid boys** in the prepubertal age group.

Theories Postulated to the downward migration of testes are:

(**Theories of testicular descent**)

1. Traction by gubernaculum
2. Differential body growth
3. Increased intraabdominal pressure
4. Epididymal differentiation theory
5. Hormonal influences (MIS, Insl.3, descendin and pituitary or placental gonadotropin deficiency)
6. Androgen dependent action of genitofemoral nerve (CGRP)
7. Presence of maternal estrogens
8. Epididymal growth factor

Risk factors:

1. Maternal obesity
2. Caesarian section
3. LBW berth weight
4. Prematurity
5. Tendency towards miscarriages, missed abortion and decreased fertility
6. Familial-14% (Multifactoral) – autosomal dominance with incomplete peneterance
7. Environmental factors: a) Diethyl stilbosterol
   b) DDT
   c) Industrial surfactants
   d) Natural phyto estenogens (Soya beans)

Diagnosis:

1. Prenatal history of Hormonal ingestion
2. Family history of UDT or hormonal disorders.
3. Previous history of descended testes (to R/O ascending tests)
4. Prior inguinal hernia surgery
5. Physical examination
   a) Presence or absence of normally developed scrotum
   b) Contralateral testicular hypertrophy (Non specific)
   c) Examination in sitting/Squatting position to look for lowermost possible position in Scroterm
   d) Evidence of intersexuality

Associated anomalies:

1. Microcephaly
2. Prune belly syndrome
3. Post-urethral valve
4. Gastroschisis/Exomphalos
5. Bladder Extrophy
6. Neural tube defects
7. Separation of epididymis and vas.

Complication:

1. Decreased fertility
2. Malignancy
3. Trauma
4. Torsion
5. Inguinal hernia
6. Psychological anxiety.

Investigation:

1. Physical examination
2. USG
3. CT Scan/MRI
4. Diagnostic laparoscopy
5. Aortography, selective gonadal arteriography and venography-not routinely used.

Indication for treatment:

1. Protection of future fertility
2. Possible prevention of malignancy and its early detection
3. Correction of associated hernias and Torsion
4. Alleviation of psychological unrest.

Management options:

1. **Hormonal**: more successful in Retractile and B/L UDT with hormonal basis.

2. **Surgery:**

    a) Standard orchiopexy (single or two stage)

    b) Laparoscopic assisted orchiopexy

    c) Orchidectomy – selected cases

    d) Testicular prosthesis following orchidectomy.

AIM OF THE STUDY

To evaluate the **histopathological** changes in **undescended** testes with relation to age and location of the testis in **testicular biopsies** taken during **orchiopexy,** which can be helpful to **prognostigate** on **future fertility**, and also to look for the presence or absence of **intra-tubular germ cell neoplasia**.

MATERIALS AND METHODS

This **prospective** study was done from **October 2005 to March 2007** over a period of 18 months, in the **Department of Pediatric surgery** with the assistance of the **Department of Pathology** the **Institute of Child Health and Hospital for Children, Madras Medical College, Chennai**.

During the above period, **testicular biopsies** in cases of **undescended testes**, either **unilateral or bilateral** undergoing **orchiopexies** were done on **21** children with **28** biopsy specimens, varying in age from **10 months-10 years**.

Inclusions criteria : All cases of **Undescended** testis

Exclusive criteria : **Retractile** and **Ectopic** testis.

The specimens were fixed in **Bouin's** solution (**saturated aqueous picric acid 75 ml. 40% formaldehyde-25ml, and glacial acetic acid-5ml**) for atleast **6 hours** for complete fixation, and subjected for **histopathological examination**.

After routine processing, sections of **4-5 micron** thickness were made and stained with **Hematoxylin** and **Eosin** (**H&E**) and **Masson Trichrome (MTS)**; viewed under microscope and analysed. **Semithin** sections were also taken and studied to look for the presence of **spermatogonia**.

Sections were analysed for the **number of seminiferous tubules**, **diameter of the seminiferous tubules**, **presence of sertoli cells with or without hyperplasia**, **presence of spermatogonia** and the **presence or absence of intra-tubular germ cell neoplasia**. **Morphometeric analysis of the tubules** were done using **image analyzer** using the Software (**Image Pro plus 6**) after measuring **50 tubules per section**. **Peritubular fibrosis, calcification, macrophages** and **hyalinization** were also analysed.

The following analysis made:

1) **Tubular fertility Index**: defined as percentage of tubules containing germ cells
2) **Mean Tubular diameter**: measured on 50 tubules per cross section using the image analyser.
3) **Sertoli cell Index**: Mean number of sertoli cells per tubule.

These indices were calculated and compared with **reference values** published by **Nistal** and **Pagnigua**, in the **Journal of Human Pathology**, **1980** and were classified into **4 types** based on these indices.

TYPE I : Minimal alteration

TYPE II : Marked germinal hypoplasia

TYPE III : Severe germinal hypoplasia

TYPE IV : Immature Sertoli cell hyperplasia with no Germ cells.

RESULTS

28 testicular biopsies were taken from **21** children undergoing orchiopexy either for **unilateral** or **bilateral** testes.

Youngest child: **10 months**

Oldest child: **10 years**

Of the **21** children, **7** had **B/L undescended testes**, totalling for **28** biopsies.

The following results were observed:

A) BASED ON TYPE:

Type	Number	Age (Years)	Location	
			Intraabdominal	Canalicular
I	6	1¼ – 6	4 (1¼; 1¼ 1½; 6)*	2 (1½; 3)*
II	-	-	-	-
III	8	1¼ -8	3 (3;3;7)*	5 (1¼;3;3;4;7)*
IV	9	10/12-10	3 (10/12; 1;7)*	6 (1¼; 1½; 1½;1½;8;10)*
Vanishing Testis	5	2¾-10	(2¾;3;5;8;10)*	

* Indicates the age of each child in that particular group.

B) **Relationship between age, location and the type:**

CANALICULAR: (n=13)

Type	Age			
	0-1 Yrs	1-2 Yrs	2-5 Yrs	5-10 Yrs
Type I	0	1	1	0
Type II	0	0	0	0
Type III	0	1	3	1
Type IV	0	4	0	2

ABDOMINAL: (N=10)

Type	Age			
	0-1 Yrs	1-2 Yrs	2-5 Yrs	5-10 Yrs
Type I	0	3	0	1
Type II	0	0	0	0
Type III	0	0	2	1
Type IV	1	1	0	1

Normal histology comprising a **Mean Tubular Diameter** of **90 or >90 microns**, **Tubular Fertility Index** of **>50%** and **Sertoli Cell Index** corresponding to the age was not observed in any of the **28 testicular biopsies**.

Among all the **28** specimens showing alterations, there were **6 Type I** lesions (**4 Intraabdominal; 2 canalicular**), **no Type II** lesion, **8 Type III** lesions (**3 intraabdominal; 5 canalicular**), **9 Type IV** lesions (**3 intraabdominal; 6 canalicular**) and **5 'Vanishing testis'** (nubbins with blind ending vas and spermatic vessels with no evidence of seminiferous tubules, leydig cells or sertoli cells) .

Hyalinization of seminiferous tubules, presence of **calcification**, **macrophage**, along with **peritubular fibrosis** were observed in **3 Type IV** lesions and none in other types.

2 cases among the **5 vanishing testis** presenting as **nubbins** showed **peritubular fibrosis** and **calcifications**.

Intracanalicular testis (palpabale) contributed to (**13 nos**); **2** were **Type I**, **5 Type III** and **6 Type IV**, and **none** in **Type II**.

Intraabdominal (Nonpalpable testis) contributed (**10 nos**), excluding the 5 Vanishing testes; 4 were **Type I**, **3 Type III** and **3 Type IV** and **no Type II** lesions.

COMPARISON AMONG THE VARIOUS AGE GROUPS

Age	Location		Type	Nos.
	Intraabdominal	Canalicular		
0-1 Yr.	1	-	IV	1
1-2 Yrs.	3	1	I	4
	-	1	III	1
	1	4	IV	5
2-5 Yrs.	-	1	I	1
	2	31	III	5
5-10 Yrs.	-	1	I	1
	1	1	III	2
	1	2	IV	3

There was **no evidence** of any tubule showing **intra-tubular germ cell neoplasia**.

DISCUSSION

A study of **Prepubertal Cryptorchid testicular biopsy** typically includes the measurement of **Mean Tubular Diameter, Tubular Fertility Index** and **Sertoli cell Index**.

They are classified as **Type I** (**minimal alterations**) with **MTD: 70 - 90 µ ; TFI > 50%** ; and with **normal number of sertoli cells** (**SCI**) per seminiferous tubule.

Type II (**marked germinal hypoplasia**) with **MTD: 60-70 µ; TFI**: **30-50%**; and **normal SCI**.

Type III (**severe germinal hypoplasia**) described as testis with **MTD: <60 µ; TFI: <30%** and **decreased sertoli cells**.

Type IV as lesions with **MTD: <60 µ; TFI <30%**; along with the presence of '**immature sertoli cell**' **hyperplasia**. '**Vanishing testis**' were described as **nubbins** of testicular tissue with only a **blind**

ending vas and **spermatic vessels** on it, with **no evidence** of either **seminiferous** tubule, **leydig** cells or **sertoli** cells.

We tried to establish a **correlation** between the **histological type** of testicular lesion and the **age** at which orchiopexy is done, as well as the **location** of the testes. All the four histological types of lesions were observed in all the age groups and in all the location from the **intraabdominal** region to the **canalicular** region from 10 **months to 10 years.**

In our study changes start occurring **as early as 10 months** from a child operated on that age showing a **type IV** lesion and these **type IV** lesions were reported in **5 out of 10 cases (50%)** between **1-2 years**, 4 being canalicular.

The severity of the type of lesion should increase as age advances in general, but our study showed only **3** children out of **9** with **type IV lesion (33.3%)**, probably because of the small study population.

Those **type IV** lesion which are found in older children in the age group of **5-10** years showed relatively more frequent presence of microcalcification, hyalinization and interstitial fibrosis.

No evidence of intra tubular germ cell neoplasia was present in any of the children.

The only **drawback** of the study was that we were not able to get **age matched** control biopsy specimens from **normal testis**. Therefore we had used the **normal measurements** published in the literature by **Nistal etal**, 1980 in Human pathology as reference values.

CONCLUSION

- In our **histopathological study** of **testicular biopsies** at the time of **orchiopexy,** it though showed that all the types of testicular lesions are present in all locations from the intraabdominal to the canalicular region, these changes start occurring **as early as 1 year of life and even earlier** for eg. (10 months in one child in our study) with more severe lesion type. Therefore the generally recommended timing of orchiopexy now being as 1 year can be reduced even to a still further lower age group.

- Classification of **prepubertal** testis by their **histologic type** could make it possible to **grade the prognosis** with regard to **fertility** in a large numbers of patients who undergo **Orchiopexy** at an earlier age.

- Such studies of **prepubertal cryptorchid biopsies** comparing them with **post pubertal testicular biopsies** in laterlife a larger number of cases definitely will help in **predicting future fertility**.

- Also in prepubertal cryptorchid testicular biopsies, it is also possible look for the presence of **intra-tubular germ cell neoplasia**.

MASTER CHART

No	AGE (Yrs)	Location		MTD (µ)	TFI (%)	SCI	TYPE
		Intraabdominal	Canalicular				
1	1¼	(L) Intraabdominal		>70	>50%	N	I
	1¼	(R) Intraabdominal		>70	>50	N	I
2	1½		(L) Canalicular	90	>50	N	I
	1½	(R) Intraabdominal		90	>50	N	I
3	3		(R) Canalicular	>60	>60	N	I
4	6		(R) Canalicular	80	>60	N	I
5	1¼		(L) Canalicular	47	<10%	Low Immature sertoli cell hyperplasia	III
	1¼		(R) Canalicular	50	<10%		IV
6	3	(L) Intraabdominal		60	<10%	N	III
	3	(R) Intraabdominal		60	<10%	N	III
7	1¼		(L) Canalicular	50	20%	N	III
	1¼		(R) Canalicular	50	20%	N	III
8	4		(L) Canalicular	52	<10%	Low	III
9	7		(L) Canalicular	<50	<10%	N	III
	7		(R) Canalicular	<50	<10%	N	III
10	10/12	(R) Intraabdominal		50	40%	Low , with Immature sertoli cell hyperplasia	IV

No	AGE (Yrs)	Location		MTD (µ)	TFI (%)	SCI	TYPE
11	1	(L) Intraabdominal		50	<10%	Low , with Immature sertoli cell hyperplasia	IV
12	1½		(R) Canalicular	50	<10%	Low, with Immature sertoli cell hyperplasia	IV
13	1½		(L) Canalicular	50	<10%	Low, with Immature sertoli cell hyperplasia	IV
14	1¼		(R) Canalicular	50	<10%	Immature sertoli cell hyperplasia	IV
15	7		(L) Canalicular	<50	<10%	Immature sertoli cell hyperplasia with Hyalanisation.	IV
16	8 8		(L) Canalicular (R) Canalicular	54 54	<10% <10%	Immature sertoli cell hyperplasia with Hyalanisation.	IV IV
17	2¾	(L) Non Palpable	Nubbins	-	-	-	Vanishing testis
18	3	(L) Non Palpable	“	-	-	-	Vanishing testis

No	AGE (Yrs)	Location		MTD (µ)	TFI (%)	SCI	TYPE
19	5	(L) Non Palpable	“	-	-	-	Vanishing testis
20	8	(L) Non Palpable		2 tubules with 100	-	-	Vanishing testis with Peritubular Fibrosis Hyalinization
21	10	(R) Non Palpable		>90 Very few	-	-	Vanishing testis with Peritubular Fibrosis Hyalinization

PROFORMA

Name: **Age:** **IP No.**

DOA: **DOS:** **DOD:**

Informant: **Reliability:**

Complaints:

1. **Palpability of testis:** **Palpable / Nonpalpable**

   Unilateral / Bilateral

2. **Scrotal development:**

3. **Evidence of Intersexuality**

4. **Prenatal History:**

   Family History

   Previous H/O **:** **Testicular descent**

   Surgery

   Hormonal treatment

   Associated anomalies

   Risk factors

   HPE: 1) MTD: 2) TFI: 3) SCI:
   4) Type:

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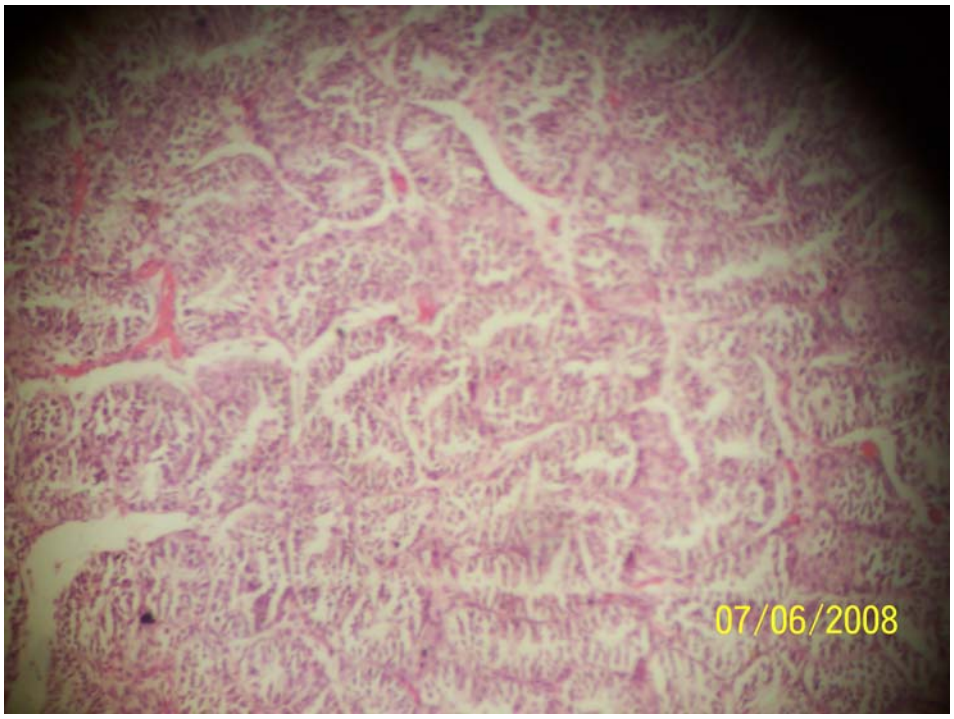
TYPE I



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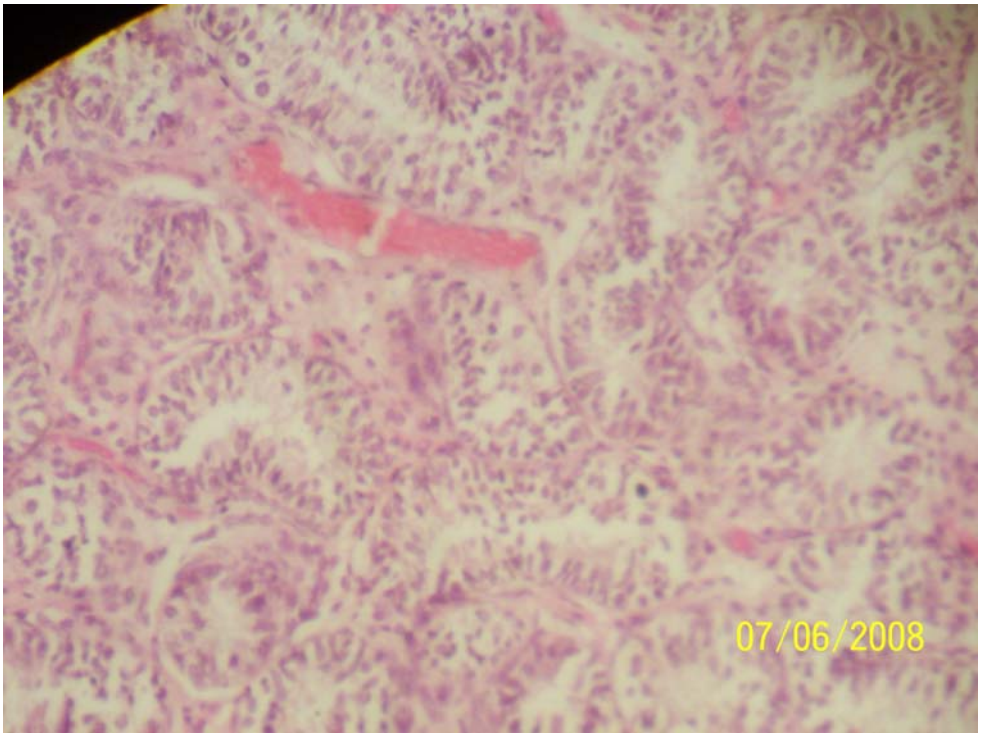

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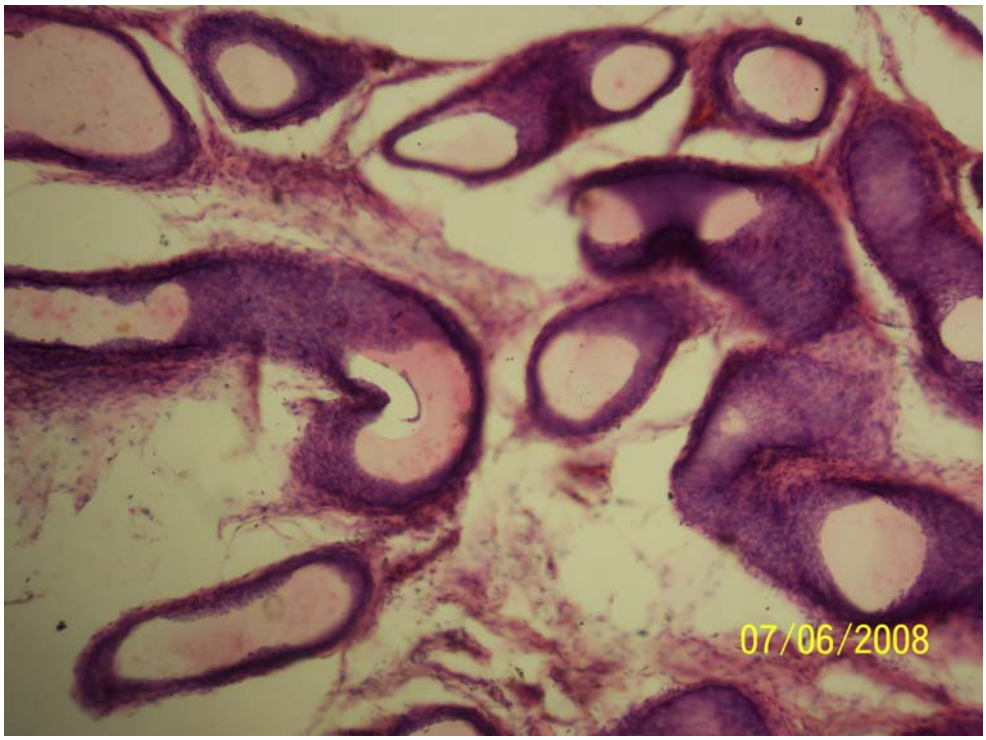

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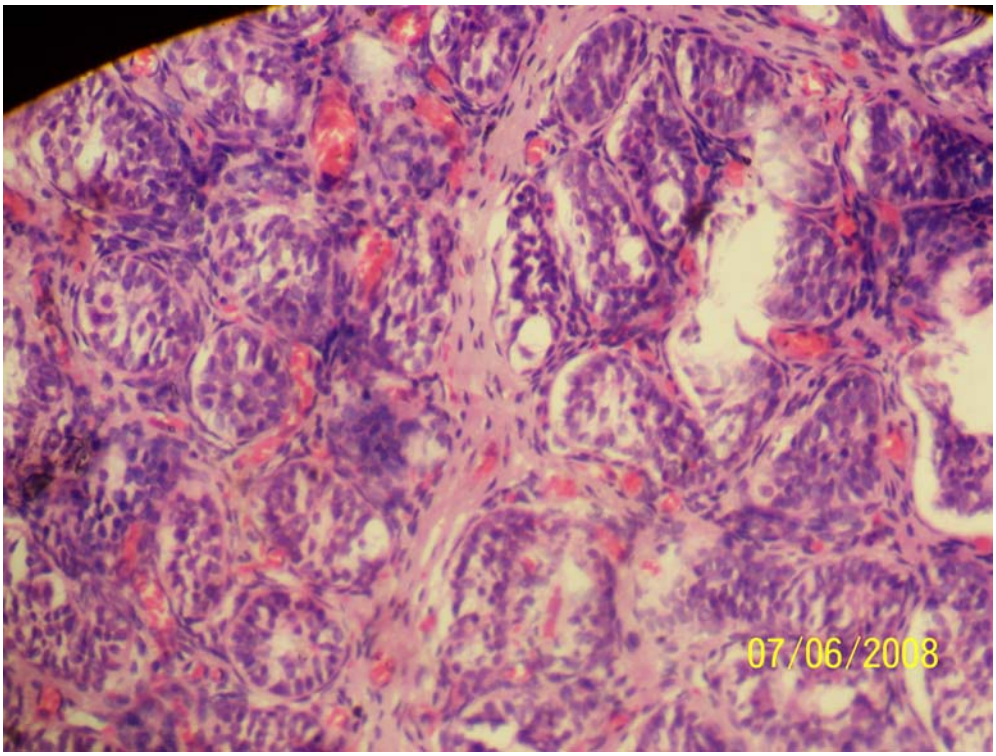

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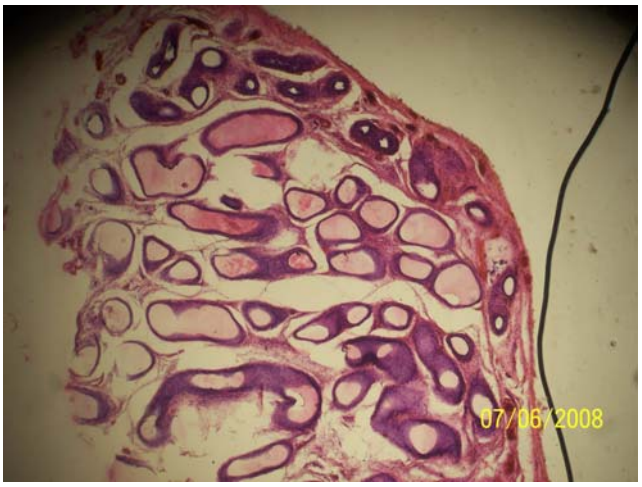
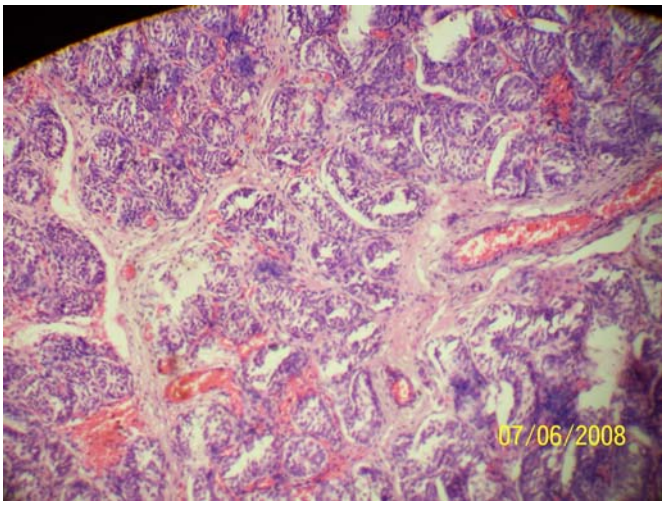


SCANNER VIEW



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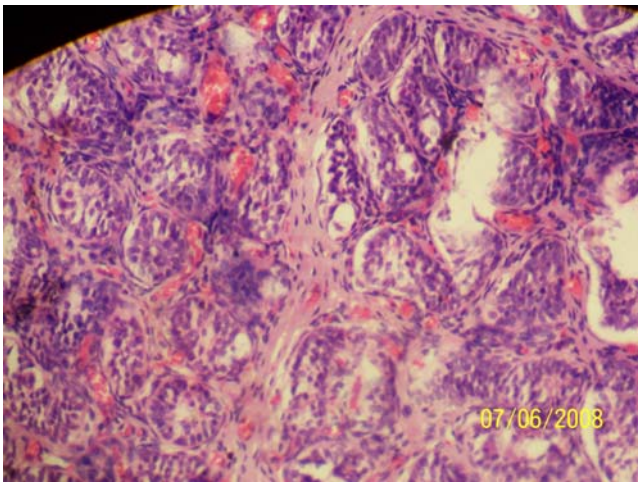

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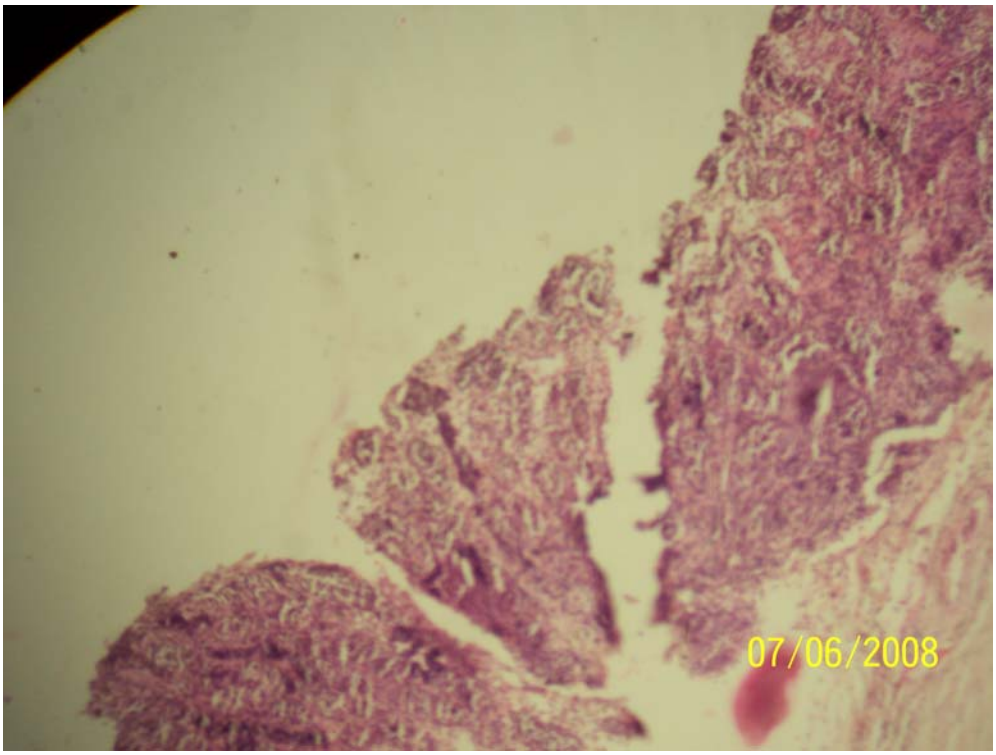

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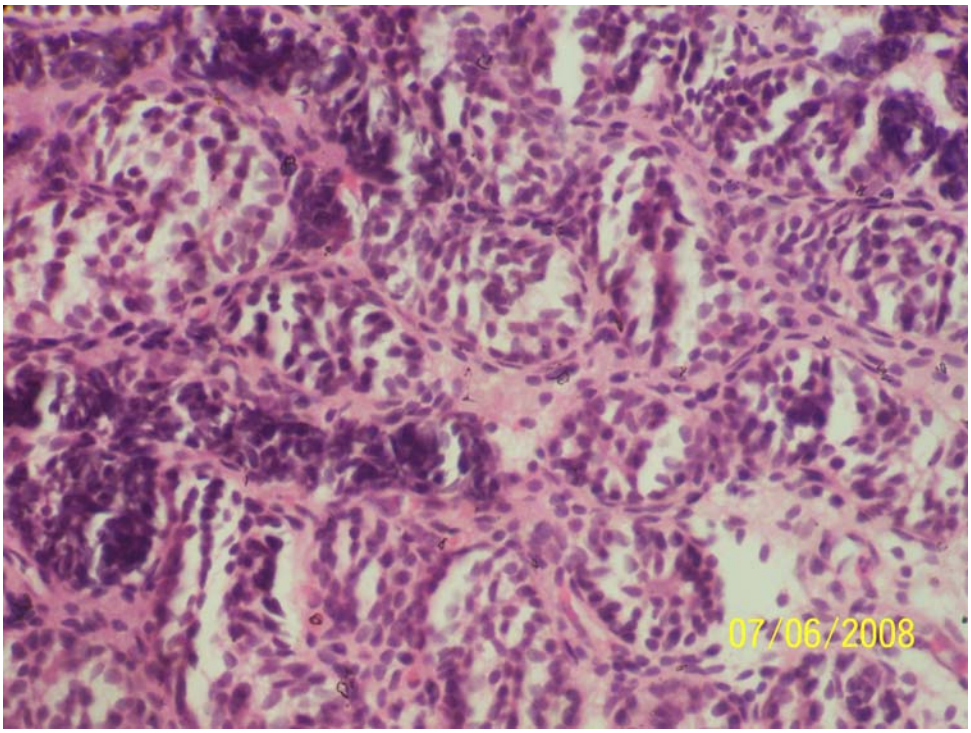

HIGH POWER VIEW

	patient caracteristics					Clinical features							CXR		CT Chest		Echo						
serial no	name	age	sex	DOS	tecnique	NYHAA Class	Duration (months)	Ascites precox	Pedal edema	Abd. discomfort	H/o AAT	JVP	ctr ratio	*CXR PE*	CT peri thick	CT Peri ca	LV Dimension(cm)	intra op cvp	post op inotropes	post op ventilation	post op nyha	Histopathology	Mortality
1	kulliammal	14	f	15/9/05	TP	III	6	pre	pre	pre	y	15	0.56	*pre*	10mm	calcific	LVF		y				
2	gopal	26	m	24/10/05	SP	III	5	pre	pre	pre	y	14	0.58	pre	11mm				y				
3	patchiammal	48	f	10-Nov	SP	IV	12	pre	pre	pre	y	16	0.58	pre	18mm				y				
4	gundammal	40	f	17/11/05	TP	III	11	pre	pre	pre	y	13	0.56	pre	16mm				y				
5	krishnaveni	21	f	22/2/05	SP	III	4	pre	pre	pre	y	13	0.6	pre	12mm				y				
6	jaganathan	26	m	12/4/2006	SP	III	5	pre	pre	pre	y	12	0.55	pre	10mm				y				
7	kaliyaperumal	19	m	25/5/06	SP	III	6	pre	pre	pre	y	13	0.56	pre	16mm				y				
8	veerakumar	19	m	10/2/2006	TP	IV	3	pre		pre	y	15	0.6	pre	14mm				y				
9	thiagarajan	18	m	7/7/2006	TP	II	2			pre	y	8	0.5		18mm	pre			y				
10	kaliappan	18	m	18/08/06	SP	IIII	24	pre	pre	pre	y	10	0.58	pre	12mm				y				
11	srinivasan	23	m	23/8/06	SP	II	3	pre	pre	pre	y	11	0.55	pre	14mm				y				
12	banupriya	14	f	24/8/06	SP	III	24	pre		pre	y	11	0.55		16mm				y				
13	shakuntala	60	f	25/10/06	TP	II	4			pre	y	9	0.55		13mm				y				
14	elishwar	36	m	9/11/2006	TP	IV	5	pre	pre	pre	y	15	0.6	pre	12mm				y				
15	mallika	35	f	20/12/06	SP	II	6	pre		pre	y	8	0.55		11mm				y				
16	niraimathi	23	f	20/2/07	SP	III	8	pre	pre	pre	y	8	0.55	pre	10mm				y				
17	robert baskar	25	m	9/3/2007	TP	III	7	pre		pre	y	13	0.58	pre	8mm				y				
18	jaya	28	f	4/4/2007	SP	II	3		pre	pre	y	9	0.55		12mm				y				
19	loganathan	50	m	18/4/07	SP	III	5	pre		pre	y	9	0.55	pre	11mm				y				
20	manohar	32	m	24/7/07	SP	III	6	pre		pre	y	10	0.5	pre	13mm				y				
21	shanthi	16	f	28/12/07	TP	IV	3	pre	pre	pre	y	15	0.6	pre	12mm				y				
22	rajendran	39	m	29/12/07	SP	III	4	pre		pre	y	14	0.58	pre	25mm	pre			y				
23	poonkodi	27	f	23/1/08	SP	IV	6	pre	pre	pre	y	11	0.6	pre	10mm				y				
24	gopal	24	m	27/2/08	SP	III	7	pre	pre	pre	y	12	0.56	pre	17mm				y				
25	nagaraj	30	m	8/4/2008	TP	II	5	pre	pre	pre	y	10	0.55	pre	16mm				y				
26	aruna	14	f	24/3/08	SP	II	5	pre		pre	y	8	0.55		18mm	pre			y				
27	sundar	29	m	6/5/2008	TP	II	6	pre		pre	y	9	0.56		25mm	pre			y				